PB82-246851

Neurobehavioral Effects of Toluene: A Review

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1981

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3
EPA-600/J-82-001	JOURNAL ARTICLE	PB82-246851

4. TITLE AND SUBTITLE	5. REPORT DATE
Neurobehavioral Effects of Toluene: A Review	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Vernon A. Benignus	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
U.S. Environmental Protection Agency, Neurotoxicology Division and Department of Psychology, University of North Carolina Medical Research Building C., #224H, Chapel Hill, North Carolina 27514	A9GA1D
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published in: Neurobehavioral Toxicology and Teratology. Vol. 3, pp. 407-415, 1981.

16. ABSTRACT

Toluene appears to produce reversible effects upon liver, renal and nervous systems. Its usual route of intake is via respiration. The nervous system appears to be the most sensitive to the effects of toluene. Although there are few studies of toluene's neurotoxicity, some tenuous results can be cited. High level toluene exposures produce incoordination, ataxia, unconsciousness and eventually, death. Lower level acute exposures in man produce dizziness, exhilaration and confusion. Activity level has been inadequately studied. Schedule controlled behaviors have been reported to produce inverted U-shaped concentration-effect curves on response rate measures. Alterations at levels as low as 150 ppm have been reported when appetitive contingencies are used. Very few studies of the nervous system have been performed at levels below 1000 ppm and most of the results were inconclusive. The TLV (threshold limit value) of toluene has been set at 100 ppm for 8 hrs. No exposures on possible groups at special risk, such as perinatal, aged or impaired subjects have been made. Few studies of reversibility of effects in the nervous system have been reported. Much more work is needed before strong conclusions can be drawn.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA-600/J-82-001
JOURNAL ARTICLE

Neurobehavioral Toxicology and Teratology, Vol. 3, pp. 407–415, 1981. Printed in the U.S.A.

Neurobehavioral Effects of Toluene: A Review

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BENIGNUS, V. A. *Neurobehavioral effects of toluene: A review.* NEUROBEHAV. TOXICOL. TERATOL. 3(4) 407–415, 1981.—Toluene appears to produce reversible effects upon liver, renal and nervous systems. Its usual route of intake is via respiration. The nervous system appears to be the most sensitive to the effects of toluene. Although there are few studies of toluene's neurotoxicity, some tenuous results can be cited. High level toluene exposures produce incoordination, ataxia, unconsciousness and eventually, death. Lower level acute exposures in man produce dizziness, exhilaration and confusion. Activity level has been inadequately studied. Schedule controlled behaviors have been reported to produce inverted U-shaped concentration-effect curves on response rate measures. Alterations at levels as low as 150 ppm have been reported when appetitive contingencies are used. Very few studies of the nervous system have been performed at levels below 1000 ppm and most of the results were inconclusive. The TLV (threshold limit value) of toluene has been set at 100 ppm for 8 hrs. No exposures on possible groups at special risk, such as perinatal, aged or impaired subjects have been made. Few studies of reversibility of effects in the nervous system have been reported. Much more work is needed before strong conclusions can be drawn.

Toluene neurotoxicity Toluene and behavior Toluene and nervous system

THE general health effects of toluene exposure, including neurobehavioral effects, have been recently reviewed [4]. The present review (a) concentrates more narrowly upon neurobehavioral data (b) reviews such data in greater detail and from a more analytic point of view and (c) updates the earlier review [4] to include more recent data.

Based upon the earlier review [4] several generalizations can be made. Toluene is a ubiquitous substance in modern industry and the home. It appears to produce no irreversible health effects on target organs outside the nervous system although temporary impairments are noted at high-level, long-term exposure. Its effects upon the nervous system are poorly explored but appear to be temporary except possibly after high-level chronic exposure. Because of the probably reversible effects of short-term toluene exposure it might be possible to study these effects in laboratory animals as well as in humans and to make possible models for generalization from non-humans to man. It seems, then, that toluene is of interest to environmental scientists because (a) it is a substance of direct, regulatory interest and (b) it might be used as a prototype for other solvents which cannot be safely studied in humans.

An attempt was made in this literature review to interpret the findings of other investigators from a consistent, critical viewpoint. If a claim was made by an author that an effect was significant, but the statistics used to support that claim were inappropriate, the statistics were corrected, where possible, and the results restated. If statistics were absent or could not be re-calculated, consideration was made of the consistency of the findings, e.g., in terms of concentration relatedness. Experimental designs, measurement methods and other techniques were similarly treated. For these reasons, the statements of the findings in the articles reviewed might not always agree exactly with the articles themselves. An effort was made to phrase the reviewer's interpretations appropriately.

Many data were converted from other units into an internally consistent nomenclature, in order to facilitate comparisons. At times results were converted from graphics to numerics to aid presentation. Inevitably in such conversions, approximations are involved. Rounding and approximations were done where no violence to the conclusions resulted.

EXPOSURE TECHNIQUES

The principal metabolism and excretion of toluene is understood [4] but the rates of uptake and elimination and pharmacokinetic details are not well documented, especially at low levels or for some species. In order to compare exposures and to interpret temporal courses of events, these details must be known.

INHALATION

Toluene typically enters the body via inhalation and most of the neurobehavioral studies reviewed herein used that route. Reports of uptake rates (to 95% of asymptote) in humans vary across investigators from about 10–80 min [2, 12, 53, 54, 55]. Uptake in mice appeared not to approach asymptote

even after 160 min [32]. The source of this wide variability is not readily apparent. Studies of uptake rate of toluene in rats at the EPA laboratories [5] indicate that asymptotic levels are approached in about 60 min. A major problem with data on toluene uptake rates as reported in the literature, is that pharmacokinetic models were not fitted so that no formal estimates of asymptotic levels or of rise times were made. When only graphic results are reported, they are difficult to evaluate because of the lack of formal estimates of parameters.

Upon termination of inhalation exposure, toluene blood levels drop precipitously, reaching 50% of maximum in about 60 min [5,32].

Brain and blood toluene levels rise at about the same rate [5,32] with brain approaching an asymptotic level of from 1.26–2.50 times that of blood [5, 31, 32, 33, 34].

By plotting data gathered from several studies, it was suggested that blood levels are approximately linearly related to exposure levels in ppm [4]. Further exploration of these data and additional data gathered in preliminary studies at EPA, indicated that blood and brain toluene levels are linear functions of exposure concentrations when they are plotted on log-log paper. Another function of log transformations is to homogenize the variances which were correlated with the means. This work is being prepared for publication.

OTHER ROUTES OF ADMINISTRATION

Toluene is also taken up into the blood when injected intraperitoneally or subcutaneously or when ingested [4]. These routes of administration have the advantages of simplicity because inhalation chamber technology is complex and the equipment is expensive and bulky. Health-effects researchers have frequently used injection [4] but few neurobehavioral studies have availed themselves of the methods.

No studies were found of the temporal course of toluene blood levels after injection or ingestion of toluene. Preliminary data from EPA studies indicates that up to 60 min might be required for maximum levels to be reached with subcutaneous injections. Blood levels appear to stay at maximum for over 60 min and then decline slowly over the course of 8–10 hr. It is possible that these results are functions of many variables and in any case are quite preliminary at present. Further study is in progress.

There is a possible disadvantage to the use of injection procedures in neurobehavioral studies since the route of administration might well influence the results. This would certainly have to be studied before extensive conclusions could be drawn. Perhaps exploratory studies could be done with injection methods and then when the pattern of effects is better understood, concentration-response functions could be studied using the inhalation method.

BEHAVIORAL EFFECTS

GENERAL OBSERVATIONS

Single Exposure

When laboratory animals are exposed via inhalation to concentrations of toluene high enough to produce unconsciousness, a typical course of events can be deduced. The initial effects seem to be excitatory, scratching and turning [21] restlessness, sneezing, visual searching and striking into the air in apparent attacks [7] and increased heart rate [33]. Prior to loss of consciousness, various motor decrements such as loss of righting in rats and mice [21, 25, 32], ataxia and paralysis in cats and rats [7,33] were observed. The times of occurrence of these symptoms were a function of exposure level. At 8,000 ppm exposures in mice, 50% decrements occurred in grip strength at 30 min, righting reflex at 40 min and rotorod performance at 14 min [32]. Seven-week-old rats exposed to 8,000 ppm showed 50% decrements in grip strength at 45 min and righting reflex at 90 min [32] and at 150 min mean performance had dropped to around 10% of maximum. Rearing behavior disappeared in rats in 8 and 1.5 min when exposed to 8,300 ppm and 26,700 ppm toluene respectively and ataxia occurred at 9 and 3 min respectively [33]. More extensive exploration of other behaviors was reported and curves were plotted [32,33] and the reader may refer to these articles for more detail. Following 10 min exposure to 8,000 ppm toluene, mice and rats reached complete recovery of function in 15–40 min, depending upon the particular task [32]. Righting reflex decrements in rats have been reported at exposure levels as low as 800 ppm but latency to onset of effects was not given [25].

Toluene exposures in humans have not been systematically studied at high levels. Male subjects [56,57] reported fatigue, headache, paresthesia, slowed pupillary reflex and confusion for toluene levels between 200 and 600 ppm and all of the above plus "hilarity", "exhilaration" and nausea for toluene levels of 600–800 ppm. Unfortunately these data were poorly quantified and repeated exposures were used. Reports of industrial accidents and of toluene abuse (similar to glue sniffing) provided data on high-level exposure in man but experimental control and documentation was usually lacking. Such effects as dizziness were reported at 200 ppm exposures and mild incoordination at exposures of 500–1,500 ppm toluene in an industrial setting [60]. During early stages of habituation to toluene, abusers were reported to exhibit dizziness and sometimes fear but during later stages of habituation, the symptoms progressed toward euphoria and indifference [43].

Exposure levels in an industrial accident were estimated at 10,000–30,000 ppm and produced unconsciousness in workers in a few minutes [26]. The exposed workers apparently were unable to judge their own danger in the above situation but whether this was due to sensory adaptation or CNS impairment was not clear. Behaviors similar to those reported for lower level experimental exposures [56, 57, 60] were also reported [26].

Repeated or Long-Term Exposure

Reports of gross symptoms due to toluene exposure in laboratory animals as a function of repeated or long-term exposures are not consistent. No cumulative alteration in motor function was found in rats or mice [32]. Alterations have been reported in latency and topography of gross motor behavior as a function of repeated exposures producing unconsciousness in rats [21] and cats [7].

Repeated exposure to high doses of toluene by abusers has been widely reported to produce dizziness and fear which progressed to euphoria and indifference, accompanied by motor weakness intention tremor and ataxia [10, 15, 17, 23, 36, 42, 43, 45, 46, 58]. Where follow-up data were available recovery was complete, with one exception [15,23], within a few months after cessation of abuse. Remitted abusers have been reported to be indistinguishable from controls

TABLE 1

EFFECTS OF TOLUENE EXPOSURES: GENERAL OBSERVATIONS AND ACTIVITY

Line Number	Description of result	Concentration related test?	Species	Lowest level (ppm) at which effect was reported	Duration of exposure	References
1.	Somatic symptoms—mood elevation, nausea	Yes	Human	200	single	[26, 56, 57, 60]
2.	Incoordination	No	Human	500–1500	single	[26,60]
3.	Ataxia—motor weakness	No	Human	Abusers	repeated	[10, 15, 17, 23, 36, 42, 45, 46, 60]
4.	Decrements in righting, grip strength, coordination	No	Rat, Mouse	800	single	[25, 32, 33]
5.	Sleep disturbances	No	Rat	1000	single	[47,48]
6.	Increased water intake	No	Rat	2000	repeated	[27]
7.	Running wheel or open field activity *NOT* affected	No	Rat	4000	repeated	[20]
8.	Open field activity did not decrease over time on subjects exposed pre- and post-natally	Yes	Mouse	400	pre- and post-natal	[24]

on such variables as personality, IQ and perceptual memory [9,28]. These data contrast with another report [16] showing decreased emotional reactivity in car painters exposed to about 30 ppm daily for up to 40 years but, unlike the remitted abusers, the painters were still being exposed at the time of testing.

ACTIVITY LEVEL

There is a striking lack of data regarding the effect of toluene upon general activity. Rats exposed to 1,000–4,000 ppm toluene for 4 hrs during sleep showed decreased slow-wave sleep time and increased paradoxical sleep at 1,000 ppm and other sleep disturbances at higher levels [47]. Similar disturbances were reported which became progressively more extreme over a 24 week period for rats exposed to 2,000 ppm toluene for 4 hr/day [48], but extensive data were not reported in that abstract. Toluene exposures of 4,000 ppm, 2 hr/day for 60 days produced no notable effects on wheel running or on open-field activity in rats [20]. An increase in "excitability" in rats was noted early in a 52-day exposure to 2,000 ppm toluene, 8 hr/day but this effect quickly disappeared [27]. These investigators did report consistently higher water intake (about 20% above controls) in the toluene-exposed rats. Mice exposed prenatally to 400 ppm in the drinking water of dams, or up to 35 days of postnatal age to the same level, showed a lack of adaptation of exploratory behavior in an open field test [24]. Postnatal exposure alone had no effect.

SUMMARY OF GENERAL BEHAVIORAL OBSERVATIONS

Table 1 lists the reports and results of the literature involving such variables as motor behavior, general activity and loss of consciousness. At low levels toluene seems to produce increased excitation and somatic symptoms (Table 1, line 1). At intermediate and high exposure levels, unconsciousness and death is preceded by locomotor and strength decrements (Table 1, lines 2–4). Sleep disturbances and increased water intake are also reported (Table 1, lines 5 and 6). Open field exploratory activity has been reported to fail to adapt under some but not all exposure conditions (Table 1, lines 7 and 8). More data are needed regarding the progress of general symptoms in toluene exposure, especially for sleeping, drinking and activity level disturbances.

PERFORMANCE AND LEARNING

The study of instrumental behavior offers advantages not available in other behavioral tests. Tasks can be devised for studying well defined classes of behavior in order to better characterize the nature of a substance's effect. Once an effect is better understood, tasks can be designed to maximize sensitivity and thus help define thresholds for effects.

Short-Term Exposures

Effects of single toluene exposures on aversively controlled behavior have been reported in a few cases. Rats were previously trained on Sidman Shock-avoidance procedure, exposed to 0, 1,000 or 3,000 ppm toluene for 4 hr, then removed from the exposure chamber and tested for 1 hr [39]. These investigators reported that while there were no increases in the number of shocks, marginal but consistant decreases in interresponse times in the 3,000 ppm group occurred. Greatest effect occurred early in the test session, just after exposure termination [41]. Bar-press shock-avoidance performance was reported to have shown "decrements" beginning at 3,200 ppm toluene exposure but documentation was very sparse [25].

Various appetitive schedules of reinforcement have also been used to evaluate toluene effects. Three rats were repeatedly exposed to 150 ppm toluene for ½, 1, 2 and 4 hr, during which the rats performed previously learned fixed-

ratio/fixed-interval (FR/FI) multiple-schedule, liquid-reinforced bar pressing [13]. No details were given about order of exposures, but sessions were separated by 3 week intervals. In the 1 hr exposure session, both FR and FI rates increased in all subjects. During the 4 hr session, all subjects showed a decrease in FR rate and 2 out of 3 a decrease in FI rate. These results could be interpreted as providing evidence for an inverted U-shaped time-and/or concentration-effects function, but, with only 3 rats in the study, these should be regarded as preliminary data.

Using a food-reinforced fixed consecutive number (FCN) schedule, data from two pigeons were reported, showing an inverted U-shaped concentration-effect curve. Various rate measures first increased beginning at 400 ppm, reached a maximum at 800 ppm toluene, then decreased beginning at 3,200 ppm [59]. Performance was evaluated after the cessation of a 4 hr exposure. Because of the small numbers of subjects these data, too, must be regarded as preliminary.

Two recent studies using mice exposed on a short term basis to toluene have reported inverted U-shaped concentration-effect curve. A DRL (differential reinforcement of low rates) schedule with appetitive reinforcement was used in one [29] while the other [14] used an FI schedule with appettive reinforcement. Exposure concentrations at maximum response rates were in the 1000-ppm region. Response rate increases were observed to be greater than those usually reported for amphetamine with similar FI schedules (Glowa, personal communication). It should be noted that in one of the above studies [29] data were collected after exposure.

Five rats were exposed to 570, 1,150, 2,300 and 4,600 ppm toluene during a 36-min session of water-reinforced responding [6]. Subjects were run on multiple FR/DRL schedules. Beginning at 574 ppm toluene, mean response rates declined during both DRL and FR. Effects were concentration-related. These results appear to contradict the inverted U-shaped concentration-effects curves reported above.

Only one study of performance was found in which humans were given 20-min exposures to toluene (100, 300, 500 and 700 ppm) [12]. Menthol crystals were used in both experimental and control subjects to mask toluene's odor. In experimental subjects, exposure levels were given in contiguous, ascending order during performance testing. Concentration-related increases in reaction time and decreases in perceptual speed were reported, beginning at 300 ppm. In this design, it was not possible to evaluate the effects of menthol exposure alone nor of any possible effects of menthol and toluene in combination.

Repeated or Long-Term Exposure

Several investigators have used shock avoidance in rats to study the effects of repeated toluene exposure. Rats were exposed to 600 ppm toluene, 4 hr/day for 9 days of acquisition and 5 days of extinction of a shuttle-box shock avoidance response [3]. The test session began 2 hr after exposure started. Acquisition was not affected but toluene-exposed rats took more trials to reach extinction. In another study using multiple goal-box avoidance responses [27] previously trained rats were exposed for 28 days to 2,000 ppm toluene for 8 hr/day during testing. After 12 more days exposure on the same regimen, 11 days of extinction were run. Again, performance was not affected but extinction was slowed. Sidman Shock-avoidance behavior was used to evaluate effects of 12 weeks of 2,000 ppm toluene exposure for 7 hr/day except weekends [40]. Performance was evaluated after exposure termination, but, no effects were obtained. Although the authors mentioned individual differences in toluene sensitivity, only anecdotal evidence was given. Results of performance during extinction were not reported.

Only one report was found concerning the effects of long-term toluene exposure on appetitively controlled behavior [20]. Selected rats were trained on continuous reinforcement (CRF) and FR-30 schedules, and then exposed for 60 days, 2 hr/day, to 4,000 ppm toluene. No differences were noted between exposed and control rats performance or extinction. Seven days after exposure to toluene was discontinued and after extinction, rats were trained on a DRL schedule. Toluene-exposed rats obtained fewer reinforcers during DRL acquisition and asymptotic performance. This result implies some effect of earlier exposure to toluene and should be investigated further.

It has been reported that humans exposed to toluene for long periods of time in a work setting perform more poorly than controls on cancellation tasks, perceptual speed, reaction time and word-digit-association tasks [37]. Personality traits were also reported to differ. Documentation in this report was extremely sparse, however, and the results are difficult to evaluate. Both IQ and emotional reactivity were reported to be lower in car painters who had been exposed to an average of 30 ppm toluene for 1–40 years [16].

SUMMARY OF BEHAVIORAL EFFECTS

The known behavioral effects of toluene are summarized in Table 2. The purpose of this Table will be to (a) try to find thresholds and (b) try to find patterns.

Patterns of Effects

Toluene appears to produce CNS anesthesia at high levels but excitatory behaviors occur, followed by motor impairments prior to unconsciousness. This is a similar pattern to general anesthetics and toluene shares other characteristics with such substances [35]. It might be of some utility to attempt to make generalizations about toluene based upon theories about CNS anesthetics but this should be done with caution since those theories are not in agreement with each other and with some of the data [11].

Under some conditions toluene exposure appears to produce response rate increases which are later (or at higher concentrations) reversed so that an inverted U-shaped concentration-effects curve occurs (see lines 1–4, Table 2). This finding is apparently a function of concentration and/or duration of exposure. It is also not clear how such findings should be interpreted. Changes in response rates, *per se*, cannot be given a simple activation or sedation interpretation [8,22]. If the conditions under which rates are increased or decreased were thoroughly explored, a pattern might emerge which could be interpreted. Perhaps some two-process hypotheses would be fruitful, such as the possibility that early rate increases are due to irritation and/or stimulation of one CNS system while response rate decreases might be due to sedative or anesthetic effects which eventually take over. Systematic work using schedule-controlled behaviors should be performed in conjunction with physiological and biochemical studies in order to provide a comprehensive account of the impact of toluene exposure.

TABLE 2

EFFECTS OF TOLUENE EXPOSURES: PERFORMANCE AND LEARNING

Line Number	Description of result	Concentration related test?	Species	Lowest level (ppm) at which effect was reported	Duration of exposure	References
1.	Increased response rate—Sidman avoidance	Yes	Rat	3000	single	[41]
2.	Increased response rate for 1 hr decreased for 4 hr-FR/FI appetitive	?	Rat	150	single	[13]
3.	Increased response rate after 400 ppm, decreased after 1600-FCN appetitive	Yes	Pigeon	400	single	[59]
4.	Increased response rate up 1000 ppm, followed by decreased rates	Yes	Mouse	—	single	[14,29]
5.	Decreased number of reinforcers FR/DRL—appetitive	Yes	Rat	574	single	[6]
6.	Slowed extinction—shuttle box avoidance	No	Rat	600	repeated	[3]
7.	Slowed extinction—multiple goal box avoidance	No	Rat	2000	repeated	[27]
8.	Decrements in DRL—appetitive	No	Rat	4000	repeated	[20]
9.	Slowed reaction time and perceptual speed	Yes	Human	300	single	[12]
10.	Slowed reaction time and perceptual speed	No	Human	?	repeated	[37]
11.	Reduced IQ and emotional reactivity	No	Human	30	repeated	[16]
12.	IQ, personality and perceptual memory *NOT* affected	No	Human	Abusers	repeated	[9,28]

Threshold

It is not necessarily logical to search through all of the above reports and pick the lowest reported effective concentration as the threshold for toluene's effect on behavior. Even if an effect can be interpreted as a decrement and if it can be assumed that the report was not a Type I error, the result must be further explored and integrated into a pattern of dose-related findings before the lowest-level effect can be meaningfully interpreted. Unfortunately, the state of the literature is not yet such that interpretation and integration can be done. Too many results have not been followed up in a systematic research program and so remain isolated findings.

Despite the isolated findings (see in Table 2), some generalizations about lowest-level effects can be hazarded. The lowest level of effects are apparently obtained in experiments testing appetitively-controlled as opposed to aversively-controlled behavior (compare line 1 with line 2–5, Table 2). Some of the aversively-controlled behavior studies did not use concentration related tests (see line 6 and 7, Table 2) thus making comparison difficult, but even so, the effects which were reported were small and the concentration was high compared to effects reported upon appetitively-controlled behaviors.

Thresholds for human exposures (lines 9–12, Table 2) are not greatly different from lowest-level effects in rats (lines 2–5, Table 2) although it is not clear how this can be interpreted. It is also not clear how exposure levels and durations can be compared across species.

Reversibility

Only one study [20] tested for behavioral effects in rats several days after termination of chronic exposure. This study showed "permanent" effects but no concentration-response information was available, nor were long-term follow-up studies done. No conclusion about irreversible behaviroal effects at any level are, therefore, possible.

Conclusion

The speculations in this section are little more than idle if they serve no further function than theorizing. There is not enough data about which to theorize. These crude generalizations might help sharpen the issues and direct the design of new studies, however. It would seem that the nature of the effect of toluene upon the nervous system ought to be explored using schedule-controlled behaviors under several levels of exposure to attempt to explain the non-linear concentration-effects curves, initially increasing rates and at higher levels decreasing rates of response. It would also be interesting to see if the peak in the increase of response rate could be brought under the control of such experimental

TABLE 3

EFFECTS OF TOLUENE EXPOSURES NEUROPHYSIOLOGICAL

Line Number	Description of result	Concentration related test?	Species	Lowest level (ppm) at which effect was reported	Duration of exposure	References
1.	EEG beta increased amplitude, hippocampal theta decreased frequency	Yes	Rat	1000	single	[47,48]
2.	Multiple EEG changes—at first excitation, later sedation	No	Cat	12,000–52,000	single and repeated	[7]
3.	Scalp EEG *NOT* affected	No	Human	200	single	[44]
4.	*In vitro* nerve-muscle preparation first increased sensitivity to stimulation, then decreased	No	—	—	single	[19]
5.	Adrenal weight increased, eosinophil count decreased	Yes	Rat	1000	single	[52]
6.	Changes in EEG spectra (unspecified)	No	Rat	2000	repeated	[48]
7.	*No* changes in EEG or nerve conduction velocity	No	Human	30	repeated	[38]
8.	Decreased convulsion threshold	No	Rat	2000	repeated	[51]

manipulations as deprivation level, task difficulty or environmental co-stressors. Discovery of ways to manipulate the shape or the points of maxima on the concentration-effects curve could help in explaining the reasons for the non-linearity. Concentration-duration-effects curves for various behaviors involving more so-called "cognitive" components compared with more vigilance-like behaviors would be informative as well.

NEUROPHYSIOLOGICAL EFFECTS

By study of the nervous system directly instead of via its end product, behavior, it might be possible to add pieces to the puzzle of mechanism by which toluene produces its effects. Quite as often, direct studies of the nervous system, being difficult to relate to behavior, raise more questions than they answer. In many cases, unfortunately, the tool takes life of its own and is no longer the servant of generality. Hopefully, however, behavioral and direct-nervous-system investigations can be used in coordinated research to lend more unity to the pattern of findings.

CNS EFFECTS

Short-Term Exposures

Rats were exposed to 0, 1,000, 2,000 and 4,000 ppm toluene for 4 hrs during their sleep cycle [47,48]. In the 2,000- and 4,000-ppm groups EEG (electroencephalogram) was spectrum analyzed during waking periods. In the 0 and 1,000 ppm groups, the same analysis was performed during slow-wave sleep. EEGs were recorded from neocortex and hippocampus. During waking, neocortical EEG exhibited a concentration-related increase in beta-frequency activity at the 2,000 and 4,000 ppm levels but other frequency bands did not dramatically change. If more concomitant behavioral data and more extensive EEG data were available, this result could imply an increased CNS activation. A similar but less dramatic increase was noted in the beta frequencies of hippocampal EEG during the same periods. The theta-wave frequency in the hippocampus decreased at 4,000 ppm during waking periods but was otherwise unaffected. Theta-wave-frequency changes have been associated with the occurrence of various motor behaviors and with reward/nonreward events but interpretation of the above data is not clear without more information on the setting and on concomitant behavioral events [30]. No dramatic EEG changes due to toluene exposure were noted during slow-wave sleep.

In another study twenty cats were exposed to toluene concentrations of 12,000–52,000 ppm for 2 min durations during which EEG was recorded in many sites [7]. Since many electrode sites were recorded with respect to the number of subjects, these results must be regarded as preliminary until some replications are carried out. During the first 90 sec of exposure 20–26 Hz spindling waves occurred in the basolateral amygdala which were probably due to sniffing and excitation [18]. There was concomitant activation in the brainstem reticular formation as evidenced by low amplitude, high frequency, desynchronized activity. After ataxia and collapse, low-frequency, high amplitude waves were recorded in the cerebellum and reticular formation, while the amygdala and neocortex leads showed high frequency synchronized waves. It was not clear how systematic these changes were since data were only qualitatively analyzed and consisted mainly of example EEG records. Many of these results are probably not specific to toluene at all, but are due to novelty or excitation during the first phase and simply signs of unconsciousness toward last. Levels of stimulation as high as this are likely to produce wide-spread, general effects which are hard to interpret.

Human volunteers were subjected to 0 and 200 ppm toluene for 6 hr after both control and experimental groups had been habituated to the recording situation [44]. Scalp EEGs

were collected and qualitatively analyzed. No systematic effects were noted, however, there were only 5 subjects per group and the exposure level was low with respect to behaviorally active concentrations. Spectrum analysis of EEG data might also have been a more sensitive technique.

An *in-vitro* nerve muscle preparation was used to study the effects of toluene in the organ bath [19]. Nerve stimulation produced muscle contraction which at first increased in sensitivity after onset of toluene. Eventually the preparation became refractory. After toluene washout, sensitivity to stimulation gradually returned. The initial increase in sensitivity during toluene exposure is reminiscent of initial excitatory effects followed by sedation which have been implied in the reports of others [7, 13, 59].

Rats exposed to 0, 100, 1,000, or 2,000 ppm toluene for 8 hr showed no change in adrenal weights during exposure but concentration-related decreases in blood eosinophil counts occurred toward the end of the exposure session [52]. Adrenal weights were, however, elevated over controls in the 2,000-ppm group 3–13 hr after cessation of exposure and in the 1,000-ppm group, 10 hrs after exposure. Eosinophil counts remained reduced until 13 hr after exposure. By 16 hr after exposure all values had returned to normal.

Repeated or Long-Term Exposures

EEG abnormalities are frequently reported among toluene abusers but since the data are usually qualitatively analyzed during different stages of recovery and under different conditions, these reports are not usually consistent. In a study involving car painters exposed from 1–40 years to an average of 30 ppm toluene daily, no differences in EEG or nerve conduction velocities were found with respect to an un-exposed group of railroad engineers [38] who were matched on several demographic parameters. these investigators did, however, observe higher incidence of diffuse EEG abnormalities in both groups with respect to clinical norms. The latter observation illustrates the importance of using carefully selected control groups. These same painters were reported to have lower intelligence test scores and lower emotional reactivity than railroad engineer control subjects [16]. No other reports of EEG effects after chronic exposure to toluene were found in the literature.

Rats were exposed to 2,000 ppm toluene, 8 hrs/day for 8 weeks, and their convulsion threshold was tested weekly by injecting Bemegride (a non-specific convulsant similar to Metrazol) [51]. After the first wk of exposure, to toluene, rats had a convulsion threshold of 80% of controls. Exposed rats remained more sensitive to the convulsant, over the exposure period, at one point showing a threshold of 70% of controls. It is not clear whether the two groups' convulsion threshold diverged since no test was made of the significance of the difference in slopes of the functions.

Increased catecholamine levels were reported in various nuclei in the median eminence and hypothalamus after exposure to 550 ppm toluene, 6 hr/day for 3 days, and 1,000 ppm toluene, 6 hr/day for 4 days [1]. The findings, however, were not concentration-related and therefore require further study. A case report on two workers chronically exposed to short bursts (5–10 min) of over 2,000 ppm toluene showed increased 17-hydroxy-corticosteroid levels [49] but the data are not easily evaluated.

PERIPHERAL NERVOUS SYSTEM EFFECTS

Peripheral nervous system effects of toluene exposure have not been extensively studied. Case reports mention ataxia, tremor, and weakness but these symptoms might all be CNS in origin.

When tail nerve conduction velocity (motor and sensory nerves) were measured in rats using 300-ppm toluene in 20 subjects exposed for 12 hr/day, 16 weeks, and 10 control rats, there was no statistically significant effect of toluene exposure after correction was made for repeated, non-independent *t*-tests [35]. Exposure levels were, to be sure, not very high with respect to behaviorally effective concentrations. Another study used 1,000 ppm exposures in rats for 12 hr/day for 16 wks and showed no effects of exposure on tail nerve conduction velocity [50]. Similar non-significant results were shown for human subjects exposed in industrial settings to 30 ppm toluene for 1–40 yr [38]. Apparently for low level toluene exposure, nerve conduction velocity is not a very sensitive measure. This measure might still have utility for higher level concentration-response functions in that such data would help understand the mechanism of toluene's effects.

SUMMARY OF NEUROPHYSIOLOGICAL EFFECTS

Studies of neurophysiological effects of toluene are scarce and preliminary in nature. Table 3 summarizes reported effects.

Results might be used to infer that toluene, when an organism is first exposed to it, is excitatory (See lines 1,2 and 4 Table 3) but that inference is weak. The inverted U-shaped concentration-response data from the schedule-controlled behavior data and the observations about toluene effects, however, support this conclusion. Data about biochemical and neuropathological results are preliminary. The findings of reduced convulsion threshold is an important result which should be followed up.

What has been said about the non-systematic nature of the behavioral research can be amplified upon in the neurophysiological data. In fact the data base is too spare to say much of anything, except that much research needs to be done.

SUMMARY AND CONCLUSIONS

The most striking feature of the body of knowledge about toluene's neurobehavioral effects is its sparsity. From the available information it would be hard to decide such basic issues as (a) what the extent or threshold of the effects are (b) whether any of the effects are permanent (c) and what the nature or mechanism of the effect is. Only tantalizing scraps of evidence are found in the literature. One of the apparently reliable statements about toluene is that at low levels or short durations of exposure, the effect is "excitatory" while at higher levels and longer durations, the effects become "depressant." It is not clear, however, why this occurs or, alternatively phrased, what variables control the threshold and shape of the inverted U-shaped concentration-response function. Even these isolated findings, indicate that neurobehavioral effects are of environmental concern, at least in some settings.

The lack of data about toluene's effects upon neurobehavioral function should not be taken as justification to pursue more of the same kind of research that is currently found in the literature. A larger heap of unrelated findings would simply be more complicated to interpret without any assurrance that the augmented data set would contain more information after factoring. What is required is a program of

coordinated studies designed to answer questions about exposure techniques, classes and patterns of effects, concentration-related effects, reversibility of effects and mechanism(s) of action.

Questions about patterns of effects and/or mechanisms of action are a matter of exploratory research and follow-up studies which refine answers and try to find experimental variables which control the concentration response functions. It is possible that such variables as task difficulty, deprivation level, environmental conditions, age of subjects and task duration could have the effect of shifting the level at which toluene produces response rate increases and decreases. Neurophysiological studies can also be useful in finding explanations for the inverted U-shaped concentration-response functions. Electrophysiological work to date, however, has usually been poor and neuropathological and neurochemical work is conspicuous by its absence.

With respect to the reversibility of toluene's effects, it is important to note that only one neurobehavioral study has used perinatal exposures. Aged or impaired populations have not been studied nor have gender comparisons been made. It would not be surprising if effects were greater and/or longer duration when engendered by perinatal exposures.

It is also important to note that toluene exposures do not occur in isolation. Individuals also are exposed to other pollutants, drugs and a host of environmental conditions such as various temperatures and humidities. It is not at all inconceivable that lower thresholds for toluene's effects would be found in studies approximating the non-laboratory setting. This should not be taken as a reccommendation to study toluene in combination with all of the above without, in the same study, also investigating the effects of each variable by itself.

It appears that toluene's major effect is on the nervous system [4]. Just what the effect is and how sensitive organisms are to this substance is still mostly a matter of research.

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